IMGN632, a CD123-Alkylating ADC Bearing a DNA-Alkylating IGN Payload, Combines Effectively with Azacitidine and Venetoclax *In Vivo*, Prolonging Survival in Preclinical Models of Human Acute Myeloid Leukemia (AML)

#1375

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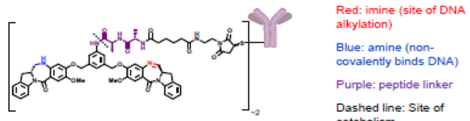
Background

IMGN632, a novel CD123-targeting ADC, demonstrates a favorable safety profile and complete remissions as a monotherapy in patients with relapsed/refractory AML and BPDCN (NCT03386513). We have previously reported the synergy of combining IMGN632 with venetoclax (BCL-2 inhibitor) (EHA, 2019, abstract #PF201). Given the recent approval of azacitidine (AZA, a hypomethylating agent) and venetoclax (VEN) in AML patients unfit for intensive chemotherapy, we investigated the combination of IMGN632 with AZA and the triple combination of IMGN632, AZA and VEN.

IMGN632 is a CD123 targeting Antibody-Drug Conjugate with a DNA alkylating payload

- IMGN632 is a novel CD123-targeting ADC
  - Composed of a humanized IgG1 antibody with high affinity to CD123.
  - Highly potent payload, DGN549, alkylates DNA without cross-linking.
  - Linker is a peptide cleaved intracellularly and is stable in circulation.
  - Conjugation is site-specific via engineered cysteines.
  - Two payload molecules per antibody.

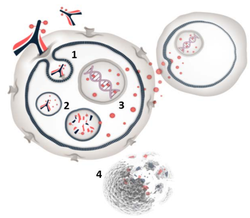


Kovtun,Yelena. ASH 2016.

ADCs with DNA alkylator promotes cell death

Antibody Drug conjugates (ADCs) with DNA alkylating payloads induce cell killing by:

1. Binding of ADC to the target and inducing internalization and uptake by the lysosomes.
2. The cytotoxic payload is released
3. Payload alkylates DNA
4. Induction of apoptotic cell death



Kovtun,Yelena. ASH 2016.

IMGN632 in combination with Azacitidine and Venetoclax induces cytotoxicity and apoptosis in MOLM-13 and MV4-11 cells

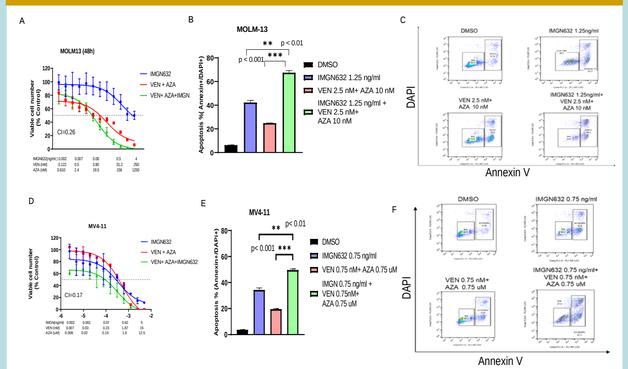


Figure 1. IMGN632 in combination with Azacitidine and Venetoclax induces cytotoxicity and apoptosis in MOLM-13 and MV4-11 cells. A. Cell viability was measured using CellTiter Glo assay. MOLM-13 parental cells were treated with IMGN632 for 48 h and VEN+AZA for 24 hours. B. MOLM-13 cells were treated with IMGN632 for 48 h and VEN+AZA for 24 h. Cells were stained with Annexin V-FITC and DAPI to determine the degree of apoptosis by flow cytometry. C. Flow cytometry analysis of MOLM-13 cells treated with IMGN632 for 48 h and VEN+AZA for 24 h. Shows the percentage of apoptosis in different treatment groups. D. Cell viability was measured using CellTiter glow assay. MV4-11 parental cells were treated with IMGN632 for 48 h and VEN+AZA for 24 h. E. MV4-11 cells were stained with Annexin V-FITC and DAPI to determine the degree of apoptosis by flow cytometry. F. Flow cytometry analysis of MV4-11 cells treated with IMGN632 for 48 h and VEN+AZA for 24 h.

IMGN632+Azacitidine+Venetoclax induces DNA damage and upregulates apoptotic proteins

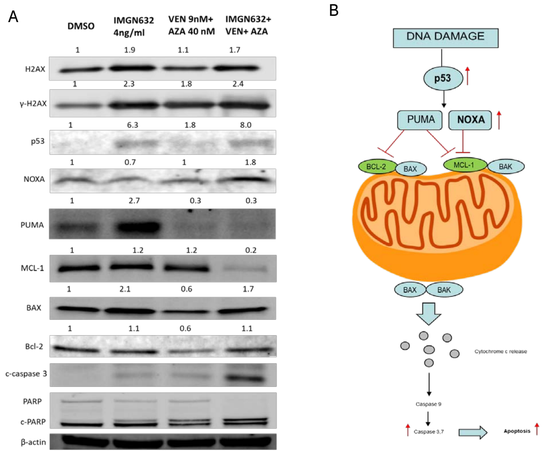
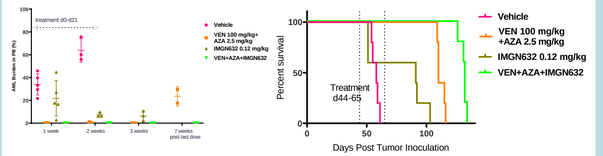


Figure 3: IMGN632+Azacitidine+venetoclax induces DNA damage and upregulates apoptotic proteins. A. MOLM-13 cells were treated with IMGN632 4ng/ml for 48 h and then venetoclax 9 nM and azacitidine 40 nm for 24 h. The collected cells were analyzed by western blotting using the indicated antibodies. B. Schematic diagram showing possible pathways by which the triple combination is inducing apoptosis.

Anti-leukemic efficacy of the triple combination in pre-clinical models of AML

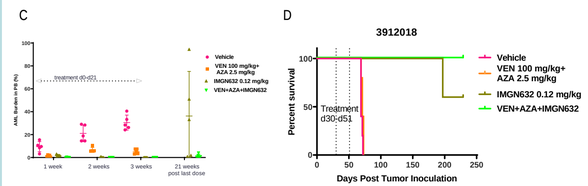
- Efficacy of the triple combination in three AML Patient-derived xenograft models (PDX)

AML PDX 4023126
Genomic Mutations: EZH2, NRAS,TET3
Average CD123 Expression: 630



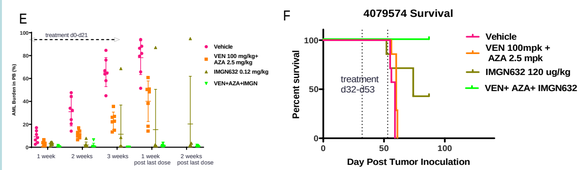
- Mice treated with VEN+AZA+IMGN632 showed a lower tumor burden than any other treatment in the PB at 7 weeks post the last dose
- The triple combination was highly active and showed the greatest median survival days of 132 days.

AML PDX 391208
Genomic Mutations: FLT3-ITD, DNMT3A, IDH1, KIT, NPM1
Average CD123 Expression: 5,154



- After 21 weeks post the last dose, the triple combination group shows a much lower tumor burden in PB compared to IMGN632 alone.
- Survival of mice treated with the VEN+AZA+IMGN632 is extended compared to the other groups.

AML PDX 4079574
Genomic Mutations: FLT3-ITD, DNMT3A, NPM1
Average CD123 Expression: 3,840

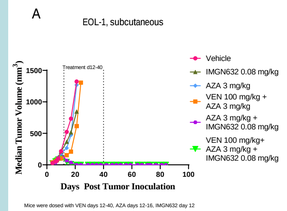


- Mice treated with the triple combination have a lower tumor burden in PB after 1 week post the last dose compared to other groups.
- Survival of mice treated with the VEN+AZA+IMGN632 is extended compared to the other groups.

Figure 3. Efficacy of VEN+AZA+IMGN632 in PDX 4023126, 3912018, and 4079574 models. Mice were randomized and grouped into four treatment groups and treated with AZA (2.5mg/kg, daily x 5), VEN (100 mg/kg, 5 days/week x3 weeks) and sub-optimal IMGN632 (0.12 mg/kg once a week x3 weeks). A, C, E. AML tumor burden was determined by measuring the percentage of hCD45+hCD123+ cells in peripheral blood. B, D, F. Survival data was estimated by Kaplan-Meier method.

- Efficacy of the triple combination VEN+AZA+IMGN632 in three cell line derived AML models

A

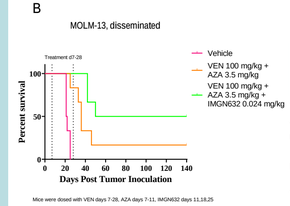


Mice were dosed with VEN days 12-40, AZA days 12-16, IMGN632 day 12

Table 1

Treatment Group	% T/C (Day 21)	Complete Regressions	Tumor Free Survivors (day 84)	Result
Vehicle	*****	2/6	1	*****
IMGN632 (0.08 mg/kg)	64	2/6	2	Inactive
AZA (3 mg/kg)	96	1/6	0	Inactive
IMGN632 (0.08 mg/kg) + AZA (3 mg/kg)	0	4/6	4	Highly Active
VEN (100 mg/kg) + AZA (3 mg/kg)	47	1/6	2	Inactive
IMGN632 (0.08 mg/kg) + VEN (100 mg/kg) + AZA (3 mg/kg)	0	5/6	5	Highly Active

B

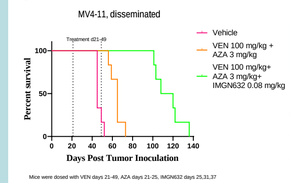


Mice were dosed with VEN days 7-28, AZA days 7-11, IMGN632 days 11,18,25

Table 2

Treatment Group	Median Survival Time (Days)	Tumor Growth Delay (Days)	% Increased Life Span	Result
Vehicle	21.5	*****	*****	*****
VEN (100 mg/kg) + AZA (3.5 mg/kg)	36	14.5	67.4	Highly Active
IMGN632 (0.024 mg/kg) + VEN (100 mg/kg) + AZA (3.5 mg/kg)	>140	>118.5	>551.2	Highly Active

C



Mice were dosed with VEN days 21-49, AZA days 21-25, IMGN632 days 25,31,37

Table 3

Treatment Group	Median Survival Time (Days)	Tumor Growth Delay (Days)	% Increased Life Span	Result
Vehicle	45	*****	*****	*****
VEN (100 mg/kg) + AZA (3 mg/kg)	65	20	44.4	Active
IMGN632 (0.08mg/kg) + VEN (100 mg/kg) + AZA (3 mg/kg)	114	69	153.3	Highly Active

Figure 4: Efficacy of the triple combination, IMGN632+Azacitidine+venetoclax in three cell line derived AML models. In the subcutaneous model, tumor volume was measured serially, complete regressions (CRs) were noted, and endpoint was determined by either clinical observations or when tumor volume reached 1000 mm^3. Tumor growth inhibition (T/C) was calculated as (median tumor volume of the treated / median tumor volume of the control) x 100%. In disseminated models, the percent Increased Life Span (%ILS) was calculated as [(T-C, tumor growth delay) / C] x 100%, where T is the median survival time (days) of a treated group and C is the median survival time (days) of the vehicle control group. Survival data was estimated by the Kaplan-Meier Method. A. Median tumor volume in EOL-1 model in indicated treatment groups. B. Survival curve of MOLM-13 model. C. Survival curve of MV4-11 model.

Conclusion

- IMGN632 in combination with AZA and VEN improved survival in patient-derived xenograft (PDX) and multiple AML xenograft models.
- IMGN632 in combination with AZA and VEN induces apoptosis, possibly by inducing p53 and upregulation of NOXA leading to apoptotic cell death.
- These data support the addition of IMGN632, a CD123 targeting ADC with a novel DNA damaging payload to standard of care AZA+VEN in AML patients.
- The combination of IMGN632 and Venetoclax and/or Azacitidine is currently being tested in a Phase1b/2 clinical trial (NCT04086264).